International Journal of Environment, Agriculture and Biotechnology
Vol-7, Issue-4; Jul-Aug, 2022

Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Peer Reviewed

Evaluation of the Phytochemical and Antibacterial Activity of Four Selected Plant Extracts against Some Pathogenic Bacteria

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Received: 27 Jul 2022; Received in revised form: 20 Aug 2022; Accepted: 25 Aug 2022; Available online: 31 Aug 2022

Abstract— *This study aimed to evaluate the phytochemical and antibacterial activity of Acanthus eminens, Celosia trigyna, Drymaria cordata, and Phytolacca dodecandra against the selected pathogenic bacteria; Two strains of Gram-positive (Staphylococcus aureus and Bacillus cereus) and three strains of Gram-negative (Escherichia coli, Salmonella typhi, and Pseudomonas aeruginosa) bacteria. The presences of phytochemicals were analyzed using the standard methods of phytochemical analysis, while the antibacterial activities were analyzed using the disc diffusion method. The results indicated the presence of terpenoids, cardiac glycosides, saponins, flavonoids, and alkaloids in the extracts of A. eminens and C. trigyna. Alkaloids, flavonoids, and phenols are present in the extract of D. cordata and P. dodecandra. Methanolic extracts of Acanthus eminens, Celosia trigyna, Drymaria cordata, and Phytolacca dodecandra were potentially effective with variable efficiency against the tested bacterial strains at a concentration of 4 mg/ml while Celosia trigyna extract was found to be the most effective with a concentration of against all tested bacterial strains. On the other hand, Phytolacca dodecandra extract was found to be effective with a concentration of against B. cereus, S. aureus, S. typhi, and P. Aeruginosa suppressing their growth with inhibition zones of 10.3, 16.7, 11.6, and 11.1 mm, respectively. Celosia trigyna and Phytolacca dodecandra methanolic extracts were the most effective plant extracts and showed bacteriostatic and bactericidal activities against the highly susceptible strains of pathogenic bacteria (Bacillus cereus, Staphylococcus aureus, and Pseudomonas aeruginosa) with MIC's ranging from 20 to 0.8 mg/ml and MBC of 4.0 and 0.16 mg/ml, respectively. These plant extracts have high potential antibacterial effects on bacterial strains tested, especially Bacillus cereus, Staphylococcus aureus, and Pseudomonas aeruginosa. They have been highly effective to be used as a natural alternative treatment to control pathogenic bacteria.*

Keywords— ***A. eminens, C. trigyna, D. cordata, P. dodecandra, phytochemicals, antibacterial activity, HPLC-UV.***

I. INTRODUCTION

In the modern world, multiple drug resistance has developed against many microbial infections due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immunosuppression, and allergic reactions. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. Plants are one of the most important sources of medicines for treating illnesses since the beginning of human civilization [1-3]. Plants in general

and high-valued medicinal plants specifically, have a long history of use as a source of cheap and effective remedy for various ailments [4]. The use of plants and herb extract in the treatment of human ailments is a very ancient art, a practice that has been passed on for generations and Scientists in Africa and other developing countries are researching local plants abundant in the continent for their possible use in traditional medicine. Plants are the richest repository of drugs for traditional medicines, modern medicines, folk medicines, pharmaceutical intermediates, and chemical entities [5-7]. It is important to mention that traditional medicinal systems are at a transitional stage in the development of modern medicines in developing countries. Therefore, the use of neglected and little-known medicinal and aromatic plants must be promoted and encouraged at regional as well as global levels for the betterment of mankind [8].

The study on medicinal plants is essential to promote the proper use of herbal medicine to determine their potential as a source for the new drugs [9]. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [10]. Drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. However, the situation is alarming in developing as well as developed countries due to the indiscriminate use of antibiotics [11].

Some researchers reported that ethanolic clove extract was potentially active against *S. aureus, Vibrio parahaemolyticus*, and *P. aeruginosa* while it was inactive against *E. coli* and *Salmonella enteritidis* [12]. Other researchers ascertained the activity of clove oil against all tested pathogenic bacteria while *Vibrio cholera, S. typhi*, and *Klebsiella* pneumonia were found to be resistant to aqueous clove extract [13, 14]. Moreover, the methanolic clove extract was reported to be potentially effective against *S. aureus*, *P. aeruginosa*, and *E. coli* with MIC ranging from 0.1 to 2.31 mg/ml [15]. The evaluation of plants for their potential application based on their medicinal properties is important for modern-day medicine as the widespread and long-term use of antibiotics has led to the emergence of multi-drug-resistant strains, besides several side effects. The adverse effects of these synthetic drugs can be overcome by using traditional or herbal formulations which are safe, efficacious, and multifunctional. Further, the development of herbal medicines based on ethnomedical leads is relatively easier in comparison to synthetic drugs [16-18]. In the present scenario of the emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from natural sources including plants. Plant and plant products play a wide range of antimicrobial properties.

II. MATERIALS AND METHODS

2.1. Plant materials Collection and Authentication. The raw material of medicinal plants *Acanthus eminens* (stems), *Celosia trigyna* (leaves), *Drymaria cordata* (leaves), *Phytolacca dodecandra* (roots) was collected from the medicinal plant's farm of Bonga University. The plant materials were washed, disinfected, rinsed with distilled water, and spread out and dried in the chemistry laboratory at room temperature for about thirty days. Dried samples of plants materials were milled into a fine powder using a high capacity grinding machine and subsequently stored separately in sterilized polythene bags in the refrigerator at the temperature of 4ºC until required for use. The plants are deposited and voulcher numbers were given by at the National Herbarium of Addis Ababa, Ethiopia. The four selected medicinal plants were authenticated by Botanist Mr. Seyoum Robo at Bonga University.

2.2. Extracts preparation. 100 g of the fine powder of plant materials were soaked in 300 ml of methanol with stirring for 72 hours, filtered through double layers of muslin, centrifuged at 9000 rpm for 10 min, and finally filtered again filtered through Whatman No1 filter paper and concentrated using a rotary evaporator at 40˚C. The resulting crude extracts were weighed and stored in the refrigerator until phytochemical screening and antimicrobial activity were carried out. The extract yields were weighted, stored in small bottles in Fridge at 5°C and their yield percentages were calculated using the following formula: Extract yield % = Q/T x 100 (where Q; the weight of extracted plants residues and T; the weight of plant powder).

2.3. Antibacterial activities of the selected plant extracts: Bacterial strains. The antibacterial potency of each plant extract was evaluated using five bacterial strains. Bacterial strains: *Staphylococcus aureus* and *Bacillus cereus* were Gram-positive and *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa* were Gram-negative bacteria. The bacterial strains were provided from the culture collection of the Mizan-Aman research center.

Table 1: The ethnobotanical data of selected medicinal plant species and their extract percentage yield.

Plant species	Family	Local name	Common name	Plant part used	Extract yield (%)
A. eminens	Acanthaceae	Phecho	Acanthus	Stems	4.63
C. trigyna	Amaranthaceae	Degicho	Woolflower	Leaves	3.27
D. cordata	Caryophyllaceae	Hakeato	Drymaria	Leaves	6.54
P. dodecandra	Phytolaccaceae	Yengamo	Endod	Roots	8.74

Table 1.1: Medicinal plants with mode of preparation used by local people from Kafa Zone, Southwest, Ethiopia. Habit: Tree (T), Herb (H), Shrub (S), Climber (C).

Synthetic Name	*Voucher name*	Habit	Parts Used	Disease and Mode of application
Acanthus eminens	*16191*	S	Stems + Leaves	Infusions of leaves of used for backache, skin diseases, cough, eye infections, wounds, pneumonia, anti-diarrhea and edema.
Celosia trigyna	*MG-S67-2005*	H	All parts	The whole parts are chopped and the sap is used for Arthritis, Diarrhea, Dysentery
Drymaria cordata	*MG-S30-2005*	H/C	Leaves	The sap is used for treating respiratory chest-ailments, colds and bronchitis.
			Above ground Part	The aboveground parts of the plant are fumigated to heal and alleviate the severe headache or migraine.
Phytolacca dodecandra	*MG-S4-2004*	S	Leaves	Infusion from leaves is used to control external parasites in livestock in general by washing their whole bodies.
			Roots	The whole parts are chopped and then mixed with water to treat Gonorrhea, rabies, anthrax.

2.4. Inoculums preparation. Each bacterial strain was sub-cultured overnight at 35 ^{0}C in an agar plate slant for 24 hrs. Individual microorganisms placed on the plate were grown into individual colonies, each a clone genetically identical to the individual ancestor organism. After the incubation, the colony of the organisms was taken and each was inoculated into 7 ml of peptone water in a bijou bottle and shook vigorously to obtain the solution homogeneity. The turbidity produced by these organisms was adjusted and used to match the turbidity standard prepared as described by [19].

2.5. Antibacterial activity of plant extract. The disk diffusion method is used to evaluate the antimicrobial activity of each plants extract. The plant extracts residues (100 mg) were re-dissolved in 5 ml of methanol, sterilized through a Millipore filter (0.22 mm) then loaded over sterile filter paper discs (8 mm in diameters) to obtain a final concentration of 10 mg/disc. 20 ml of an agar plate medium was poured into sterile petri-dishes followed with 30 ml of seeded medium previously inoculated with bacterial suspension (100 ml of medium/1 ml of 10^7 MPN) to attain 10^5 MPN/ml of medium. Sterile filter paper discs loaded with plant extract concentration of (10 mg/ml) were placed on the top of an agar plate. Filter paper discs loaded with 5 mg of Gentamycin were used as a positive control. The plates were kept in the fridge at 5 ^{0}C for 2 hrs to permit plant extracts diffusion then incubated at 35 ^{0}C for 24 hrs. The existences of inhibition zones were measured with the help of a template and the diameter of the zone of inhibition was determining the effectiveness of the antibiotic. The large diameter indicated the sensitivity of the bacterium to the antibiotic. The zone sizes were compared to a standardized chart to determine the

bacterium sensitivity, resistance, and intermediate sensitivity to that of antibiotics.

2.6. Determination of minimum inhibitory concentrations (MIC's) of the effective plant extract. Minimum inhibitory concentrations (MICs) are the lowest concentration of an antimicrobial that inhibited the visible growth of a microorganism after overnight incubation. The MIC of the selected plant extracts was carried out using a disc diffusion method and evaluating the resistivity of bacterial strains. Different concentrations of the effective plant extracts (100, 20, 4, 0.8, 0.16, and 0.32 mg/ml) were prepared separately by dissolving 200 mg in 100 ml of methanol, sterilized. The most effective extracts of plants that exhibited a strong antibacterial activity at 10 mg/ml were manipulated to determine their minimum inhibitory concentrations. 1 ml of the standardized inoculums from peptone water was then inoculated into the solution in the test tubes. These were all incubated at 37˚C for 24 hrs and observed for turbidity of growth. The lowest concentrations which showed no turbidity in the test tubes were recorded as the MIC.

2.7. Determination of minimum bactericidal concentrations (MBC's) of the effective plant extract. The minimum bactericidal concentration is the lowest concentration of a substance that prevents the growth of an organism after subculture onto antibiotic-free media or the concentration of plant extract that did not exhibit any bacterial growth on the freshly inoculated agar plates. Agar plates were incubated at the temperature of 37 ^{0}C for 24 hours then examined for bacterial growth corresponding to plant extracts concentration.

III. RESULTS AND DISCUSSION

3.1. Plants extraction yield: The ethnobotanical data of the employed plants and their extract percentage yield are illustrated in Table 1. The extract of 100 g of each dried plant material with methanol yielded plant extracts residues ranging from 2.29 to 6.12 g. The highest yield of plant extract was obtained from *Phytolacca dodecandra* (6.12 g) followed by *Drymaria cordota* (4.58 g) while *Celosia tigyna* (2.29 g) give the lowest extract yield, respectively.

Extract yield % = Q/T x 100 (where Q; the weight of extracted plants residues and T; the weight of plant powder).

3.2. Phytochemical Screening: The two extracts were screened for the presence of major phytochemicals using standard qualitative methods as described previously [20-22]. Each plant extracts were screened for the presence of terpenoids, flavonoids, saponins, tannins, alkaloids, fatty acids, steroids, phenols, cardiac glycosides, anthraquinones, and phlobatannis as outlined below:

Test for Phenols: 0.5 ml of the extract, 5 ml of Folin Ciocalteu reagent, and 4 ml of aqueous sodium carbonate were added. The appearance of blue color indicates the presence of phenols.

Test for Saponins: To 2 ml of the extract, 2 ml of distilled water was added and it was agitated in a test tube for 5 minutes. The formation of foams indicates the presence of saponins.

Test for Tannins: 4 drops of 0.1% ferric chloride were added to 2 ml of the extract, a brownish-green or blue-black coloration indicated the presence of tannins.

Test for Alkaloids: To 2 ml of the extract, 2 ml of 10% hydrochloric acid was added. To the acidic medium, 1 ml Hager's reagent (saturated picric acid solution) was added. The presence of alkaloids is confirmed by the formation of a yellow-colored precipitate.

Test for Anthraquinones: 2 ml of the extract was boiled with 5ml of 10% hydrochloric acid for 3 minutes and 5 ml of chloroform was added. 5 drops of 10% ammonia were further added. A rose-pink coloration indicates the presence of anthraquinones or a positive result.

Test for Glycosides: 2 ml of acetic acid was added to 2 ml of the extract. The mixture was cooled in a cold water bath and 2 ml of concentrated H_2SO_4 was then added, color development from blue to bluish-green indicates the presence of glycosides.

Test for Flavonoids: 2 ml of 10% Sodium hydroxide was added to 2 ml of the extract in a test tube. An intense yellow color was formed which turned colorless upon the addition of 2 ml of dilute hydrochloric acid indicating the presence of flavonoids.

Test for Phlobatannins: 2 ml of the extract were boiled with 1% aqueous hydrochloric acid. The formation of a red precipitate indicates the presence of Phlobatannins.

Test for Terpenoids: 5 ml of the extract was mixed in 2 ml of chloroform and 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish-brown coloration at the interface indicates the presence of terpenoids.

Test for Steroids: 2 ml of extract were dissolved in 10 ml of chloroform and then 10 ml of concentrated sulphuric acid was added by the side of the test tube. The upper layer turned red whereas the sulphuric acid layer turned yellow with green fluorescence. This indicates the presence of steroids.

The result for the phytochemicals screening tests (analysis) of the methanolic extracts of *Acanthus eminens*, *Celosia*

trigyna, *Drymaria cordata*, and *Phytolacca dodecandra* is shown in Table 2. While Table 3 represents the results for the antibacterial activity of the extracts of the above four selected medicinal plants against the test bacteria.

Table 2: Phytochemical presents in the methanol extracts of Acanthus eminens, Celosia trigyna, Drymaria cordata, and Phytolacca dodecandra.

Phytochemicals	*Acanthus eminens*	*Celosia Trigyna*	*Drymaria cordata*	*Phytolacca dodecandra*
Cardiac glycoside	++	+	-	-
Saponins	+++	+++	+	++
Flavonoids	+++	++	+++	++
Alkaloids	++	+++	+++	+++
Steroids	+	+++	-	+
Terpenoids	+++	+++	-	++
Phenols	++	++	+	+
Anthraquinones	-	+	-	-
Tannins	-	+	+	+

Note: +: slightly present; ++: moderately present; +++: highly present; -: not detected.

The result for the phytochemical analysis is presented in table 2. The result revealed the presence of different phytochemicals in the extract of methanol solvent. The results show that terpenoids, saponins, and flavonoids are highly present, cardiac glycoside, alkaloids, and phenols moderately present, and anthraquinones and tannins not detected in the extracts of stem bark of *Acanthus eminens*. Phytochemicals such as saponins, alkaloids, steroids, and terpenoids are highly present, flavonoids and phenols are moderately present, and cardiac glycoside, anthraquinones, and tannins are slightly present in the extracts of leaves of *Celosia trigyna.* In the leaves extract of *Drymaria cordata*, phytochemicals such as flavonoids and alkaloids are highly present, saponins, tannins, and phenols are slightly present, and cardiac glycoside, steroids, terpenoids, and anthraquinones are not detected. Finally, alkaloids are highly present, saponins, flavonoids, and terpenoids are moderately present, steroids phenols and tannins slightly present, whereas cardiac glycoside and anthraquinones are not detected in the root extract of *Phytolacca dodecandra*. In line with the present study, [23] reported that phytochemicals such as tannins, saponins, flavonoids, and alkaloids are bioactive compounds that have an extensive range of beneficial pharmacological effects like; antimicrobial, antihypertensive, antioxidant, anti-inflammatory, anticancer, and anti-diabetic activities, in addition to alleviating hypercholesterolemia.

3.3. Antibacterial activity of plant extract. Four plant species were investigated to evaluate the antibacterial activity of extracts against pathogenic bacteria including two strains of Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and three strains of Gram-negative bacteria (*Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*) using the disc diffusion method. Evaluation of the antibacterial activity of these plant extracts was recorded in Table 3 and Figure 1. The results revealed that all plant extracts were potentially effective in suppressing microbial growth of pathogenic bacteria with variable potency. *Celosia trigyna* was the most effective extract retarding microbial growth of all Gram-positive and Gram-negative bacteria tested pathogenic bacteria at concentration of 4 mg/ml while extract of *Drymaria cordata* was effective only against *Staphylococcus aureus*. *Phytolacca dodecandra* exhibited an inhibitory effect against four of the pathogenic strains (*Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa)* whereas *Acanthus eminens* was effective against three of the pathogenic bacteria (*Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa*). Results of antibacterial activity of the four plant extracts can be suggested that both *Celosia trigyna* and *Phytolacca dodecandra* plant extracts were the most effective extracts and showed strong antibacterial activity against pathogenic bacteria. The two Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi)* were relatively the most resistant strain to plant extracts whereas two Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and one Gram-negative bacteria (*Pseudomonas aeruginosa*) were the most susceptible strains to the extracted plants. Hence, experiments were conducted to determine their minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) against the most susceptible bacterial

strains (*Bacillus cereus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*). In coherent with this finding, [24] reported that significant anti-bacterial activity of *C. longa* extract against two pathogenic bacterial strains. The results of MIC and MBC of *C. longa* extract demonstrated promising antibacterial activity of *C. longa* rhizome.

Table 3: Antibacterial screening test of methanolic plants extract (4 mg/ml) against some pathogenic bacteria.

Plant species	Inhibition zones (mm)				
	Gram (+ve) pathogenic bacteria		Gram (-ve) pathogenic bacteria		
	B. cereus	*S. aureus*	*E. coli*	*S. typhi*	*P. aeruginosa*
A. eminens	9.7 ± 0.43	0.0 ± 0.0	12.5 ± 0.54	0.0 ± 0.0	10.7 ± 0.65
C. trigyna	11.2 ± 0.53	17.2± 0.12	14.2 ± 0.34	12.8 ± 0.15	14.3 ± 0.37
D. cordata	0.0 ± 0.0	7.7 ± 0.27	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
P. dodecandra	10.3 ± 0.25	16.7± 0.42	0.0 ± 0.0	11.6 ± 0.13	11.1 ± 0.21
Gentamycin(5mg)	12.6 ± 0.18	19.4 ± 0.21	14.6 ± 0.44	16.5 ± 0.37	11.3 ± 0.29

Data are means of three replicates (n = 3) ± standard error.

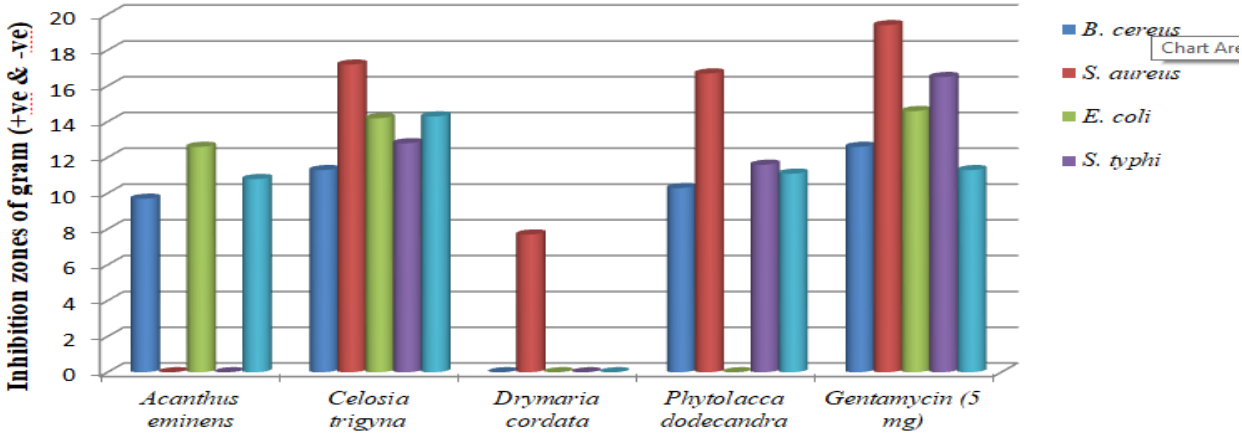

Fig.1: Methanolic plants extract against pathogenic bacteria and positive control.

3.4. Minimum inhibitory concentrations (MIC's) of the effective plant extract. The MIC and MBC of the most effective plant extracts (*Celosia trigyna* and *Phytolacca dodecandra*) were employed by the disc diffusion method to evaluate their bacteriostatic and bactericidal properties. The concentration effect of the effective plant extracts was reported in Table 4 and illustrated in Figure 2. An inhibitory effect of *C. trigyna* extract started at 20 mg/ml with inhibition zones of 8.9, 10.1, and 7.7 mm against *Bacillus cereus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* while extract of *P. dodecandra* suppressed bacterial growth of these strains at concentration of 0.8 mg/ml with inhibition zones of 16.8, 13.9 and 13.4 mm, respectively. This findings were in accordance with those reported in a work by Jawhari et al. that the inhibition zone diameters of extracts studied ranged from 5.5 to 15.65 mm, and the highest inhibition zone values against pathogens of medical importance such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumonia* were 15.65, 15, and 15.3 mm, respectively [25].

Table 4: MIC's of the most effective plant extract against Bacillus cereus, Staphylococcus aureus and Pseudomonas aeruginosa.

Plant extract	Conc. mg/ml	Inhibition zones (mm)		
		Gram (+ve) bacteria		Gram (-ve) bacteria
		B. cereus	*S. aureus*	*P. aeruginosa*
C. trigyna	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	20	8.9 ± 0.31	10.1 ± 0.95	7.7 ± 0.46

	4	11.6 ± 0.44	17.5 ± 0.23	11.7 ± 0.79
	0.8	19.4 ± 0.86	18.3 ± 0.67	17.1 ± 0.12
	0.16	22.7 ± 0.39	20.5 ± 0.36	19.3 ± 0.23
	0.032	25.9 ± 0.12	24.3 ± 0.91	21.7 ± 0.65
P. dodecandra	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	20	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.8	16.7 ± 0.85	13.9 ± 043	13.6 ± 0.79
	0.16	18.9 ± 0.13	16.5 ± 0.74	16.3 ± 021
	0.032	20.5 ± 0.46	18.7 ± 0.21	17.4 ± 0.37

3.5. Minimum bactericidal concentrations (MBC's) of the effective plant extract. The minimum bactericidal concentration was confirmed by the absence of bacterial growth of the tested strains streaked from the inhibition zone corresponding to their lowest minimum inhibitory concentrations. *C. trigyna* extract showed potentially bactericidal activity against the tested pathogenic bacteria (*Bacillus cereus, Staphylococcus aureus*, and *Pseudomonas aeruginosa*) with MBC of 4 mg/ml while MBC of *P. dodecandra* extracts reached 0.16 mg/ml except for *Pseudomonas aeruginosa* which was less sensitive and its minimal bactericidal concentration reached to 0.032 mg/ml. The results of MIC and MBC of the effective plant extracts suggested that *Celosia trigyna* and *Phytolacca dodecandra* can be used to control and prevent pathogenic bacteria. *Celosia trigyna* extract suppresses microbial growth of all tested bacterial strains followed by an extract of *Phytolacca dodecandra* which appear to be potentially effective against three bacterial strains or pathogenic bacteria (*Bacillus cereus, Staphylococcus aureus*, and *Pseudomonas aeruginosa*) and less effective against two of them (*Escherichia coli*, *Salmonella typhi*). A great variation in MIC of *Celosia trigyna* extract demonstrated in several investigations may be due to considerable variation in their method of extraction, constituents as well as bacterial strains used. The difference value in minimum inhibitory concentrations of the plant extracts has happened from the variation of secondary metabolites and volatile nature of their constituents. In line with this study, [24, 26], reported that various biological activities of plant extracts are believed to be due to the presence of bioactive compounds. They explained that these plant secondary metabolites are nutritional constituents which are present in very tiny amounts in plants and have the potential for influencing the physiological and cellular activities after consuming them.

Celosia trigyna extract was found to be the most effective with a concentration of (4 mg/ml) against all tested bacterial strains. On the other hand, *Phytolacca dodecandra* extract was found to be effective with a concentration of (4 mg/ml) against *B. cereus, S. aureus, S. typhi*, and *P. Aeruginosa* suppressing their growth with inhibition zones of 10.3, 16.7, 11.6, and 11.1 mm, respectively. These results are in accordance with that of [12, 23]. Some researchers have suggested that antimicrobial components of the plant extracts (terpenoid, alkaloid, and phenolic compounds) interact with enzymes and proteins of the microbial cell membrane causing its disruption to disperse a flux of protons towards the cell exterior which induces cell death or may inhibit enzymes necessary for amino acids biosynthesis [24, 25]. Other researchers attributed the inhibitory effect of these plant extracts to hydrophobicity characters of these plants extract which enable them to react with protein of microbial cell membrane and mitochondria disturbing their structures and changing their permeability. It has been reported that the relationship between a zone of inhibition and MIC values may be greatly affected by the composition of crude extracts that are a mixture of phytoconstituents which may influence the diffusion power of the active constituents, and the different levels of intrinsic tolerance of test strains to antimicrobials which can differ MIC values from one isolate to another [26-28].

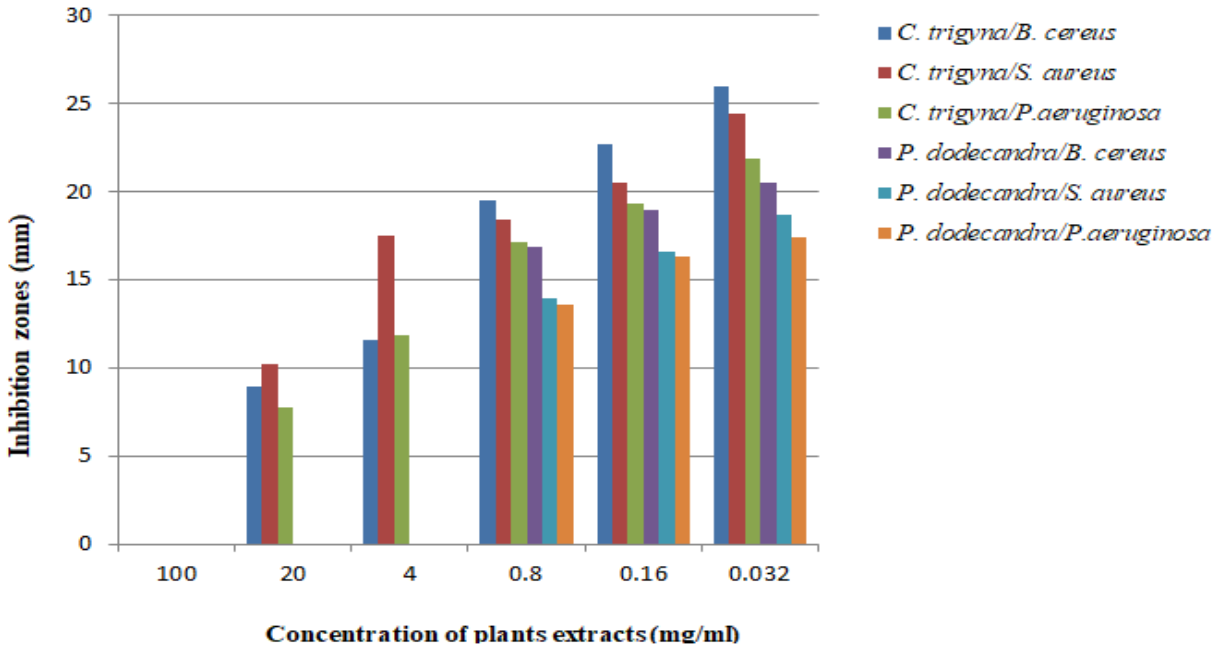

Fig.2: MIC of the most effective plant extract against B. cereus, S. aureus, and P. aeruginosa

In the present study, extracts of *Celosia trigyna* leaves and *Phytolacca dodecandra* roots have the most effective against four pathogenic bacterial strains like *B. cereus*, *S. aureus*, *S. typhi*, and *P. aeruginosa* whereas extracts of *Drymaria cordata* leaves and *Acanthus eminens* stems have indicated practically low activities against pathogenic bacteria. The observed activities of these extracts were relatively similar to other works. Thus, n-butanol leaves extracts of *Cassia angustifolia* exhibited maximum zone of inhibition against *Staphylococcus aureus* (17.0 mm), *Salmonella typhi* (12.0 mm), and *Klebsiella pneumoniae* (10.0 mm); while, methanol extracts have not shown any activity against both the isolates. MICs values of leaf methanol extract of *C. angustifolia* exhibited stronger activity against K. pneumonia and E. coli (0.62 and 1.25 mg/mL, respectively) [29, 30].

Phytochemical Analysis: The HPLC-UV chromatogram of four selected plants Me. Ext is shown in Figure 3. Seven phytochemicals were identified from *C. trigyna* leave; four phytochemicals from *P. dodecandra* root, five phytochemicals from *D. cordata* leave and four phytochemicals from *A. eminens* stem methanol extract when compared to the standard chromatogram. The identified compounds from the HPLC chromatogram as shown in Figure 3 were pheophytin, chondrillasterol acetate, chondrillasterol, carotenoid, lutein, ethinyl estradiol and drospirenone isolated from *C. trigyna* leave methanol extract. Citronellal, cardinene, nerolidol and neryl acetate were isolated from *P. dodecandra* root methanol Extract. Stigmasterol, cerebroside, glucocerebroside, monogalactosyldiacylglycerol and digalactosyldiacglycerol were isolated from *D. cordata* leave methanol Extract. Isopulegol, borneol, caryophyllene and linalool were isolated from *A. eminens* stem methanol extract. The respective peak position, retention time and concentration of identified phytochemicals are given in Table 5.

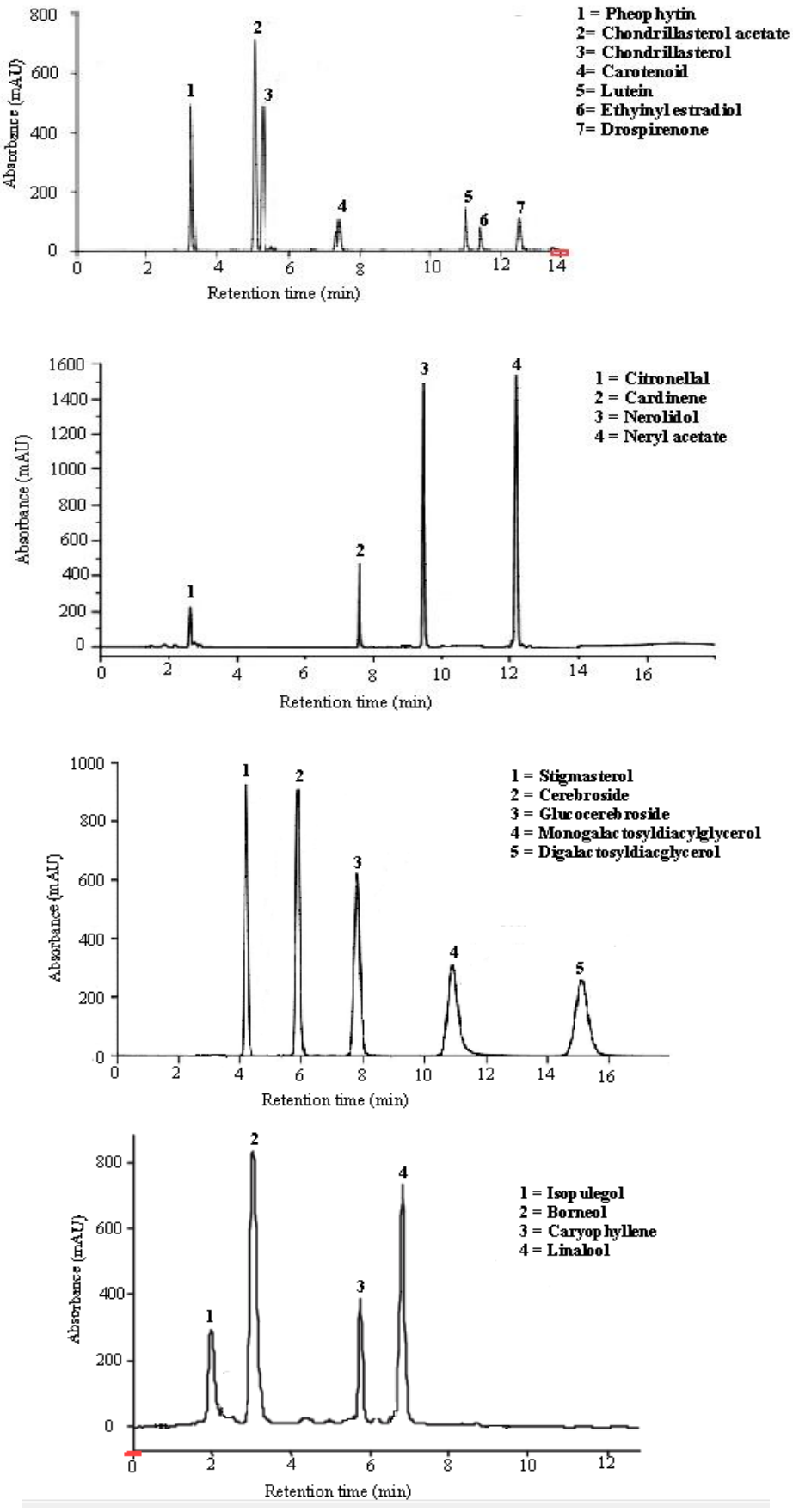

Fig.3: HPLC-UV chromatogram of C. trigyna leave, P. dodecandra root, D. cordata leave and A. eminens stem Me. Ext

Table 5: Identified phytochemicals in C. trigyna leave, P. dodecandra root, D. cordata leave and A. eminens stem Me. Ext.

Peak	Retention Time (min)	Phytochemicals	Peak area	Concentration (µg/ml)
C. trigyna leave Me. Ext				
1	13.54	Pheophytin	7.23	0.5427
2	32.65	Chondrillasterol acetate	2.21	0.0034
3	18.77	Chondrillasterol	0.45	4.3134
4	25.82	Carotenoid	0.68	0.0231
5	10.37	Lutein	0.97	0.7254
6	19.58	Ethinyl estradiol	0.32	1.3125
7	11.54	Drospirenone	0.77	2.7326
P. dodecandra root Me. Ext				
1	11.98	Citronellal	85.06	3.3412
2	24.83	Cardinene	0.78	0.5754
3	25.27	Nerolidol	0.54	0.0043
4	19.21	Neryl acetate	0.75	2.1432
D. cordata leave Me. Ext				
1	16.43	Stigmasterol	5.67	0.7489
2	26.52	Cerebroside	0.69	0.1227
3	12.93	Glucocerebroside	0.34	2.1539
4	11.57	Monogalactosyldiacylglycerol	0.72	0.0856
5	21.34	Digalactosyldiacglycerol	0.89	1.5328
A. eminens stem Me. Ext				
1	11.53	Isopulegol	4.56	5.5321
2	13.25	Borneol	0.48	0.9234
3	20.62	Caryophyllene	0.79	0.0069
4	11.46	Linalool	0.63	1.2954

IV. CONCLUSION

The findings of this study indicate about methanolic extracts of four selected medicinal plants have high potential antibacterial activity against the different pathogenic bacterial strains. This activity supports their use in the treatment of infections caused by such resistant bacteria. The plant extracts which proved to be potentially effective are *Celosia trigyna* and *Phytolacca dodecandra* those can be used as a natural alternative for the treatment of pathogenic microbes, this has led to the search for new antimicrobial agents mainly among plant extracts to discover new chemical structures according to modern phytochemistry. The extract of those two plants has potential antibacterial effects on bacterial strains tested, especially *Bacillus cereus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Their antibacterial activity was confirmed by evaluation of both diameters of inhibition zones and minimal inhibitory concentrations.

DATA AVAILABILITY

The data are available from the corresponding author upon request.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that there is no conflicting interest.

ACKNOWLEDGMENTS

The authors acknowledge all the technical support offered by microbiology staff from the Department of Biology and Plant Science. We authors also thank Bonga University for financial support.

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